

In the Claims:

1. (previously presented) A method, comprising:

a) providing a plasma sample from a subject, said plasma sample comprising genomic DNA, wherein said genomic DNA comprises a plurality of promoters from different genes;

b) digesting said genomic DNA in said plasma sample with a methylation-sensitive restriction enzyme under conditions such that unmethylated CpG islands in said promoters are cleaved while methylated CpG islands in said promoters are not cleaved;

c) contacting said plasma sample with at least five different pairs of gene specific primers, wherein said gene specific primers are configured to hybridize to said genomic DNA and amplify five different promoters from at least five different genes, and wherein said contacting is under conditions such that fragments of said plurality of promoters comprising uncleaved CpG islands are amplified, while cleaved promoters comprising cleaved CpG islands are not amplified; and

d) detecting the presence or absence of DNA methylation in each of said plurality of promoters based on the amplification, or lack of amplification, of said fragments to generate a methylation profile for said subject.

2. (previously presented) The method of Claim 1, wherein said method further comprises comparing said methylation profile to more or more standard methylation profiles, wherein said standard methylation profiles are selected from the group consisting of methylation profiles of non-cancerous samples and methylation profiles of cancerous samples.

3. (currently amended) A method of characterizing cancer, comprising:

a) providing a biological sample from a subject diagnosed with breast cancer, said biological sample comprising genomic DNA; and

b) detecting the presence or absence of DNA methylation in DAPK and at least one additional gene selected from the group consisting of: GSTP, p15, MDR1, Progesterone Receptor, Calcitonin, RIZ1, and RARBeta to generate a profile, thereby characterizing said breast cancer in said subject.

4. (currently amended) The method of claim 3, further comprising the step of detecting the presence or absence of DNA methylation in at least one additional gene selected from the group consisting of S100A2, SRBC, BRCA1, HIN1, Cyclin D2, TMS1, HIC-1, hMLH1, E-cadherin, 14-3-3sigma, GSTP, p15, MDR1, Calcitonin, RIZ1, RARbeta, and MDGI.

5. (original) The method of claim 3, wherein said characterizing cancer comprises detecting the presence or absence of chemotherapy resistant cancer.

6. (original) The method of claim 5, wherein said chemotherapy is a nonsteroidal selective estrogen receptor modulator.

7. (original) The method of claim 3, wherein said characterizing cancer comprises determining a chance of disease-free survival.

8. (original) The method of claim 3, wherein said characterizing cancer comprises determining the risk of developing metastatic disease.

9. (original) The method of claim 3, wherein said characterizing cancer comprises monitoring disease progression in said subject.

10. (original) The method of claim 3, wherein said biological sample is a biopsy sample.

11. (previously presented) The method of claim 3, wherein said biological sample is a plasma sample.

12. (original) The method of claim 3, wherein said DNA methylation comprises CpG methylation.

13. (original) The method of claim 3, wherein said detecting the presence or absence of DNA methylation comprises the digestion of said genomic DNA with a methylation-sensitive

restriction enzyme followed by multiplexed amplification of gene-specific DNA fragments with CpG islands.

14. (original) The method of claim 13, wherein said methylation-sensitive restriction enzyme comprises *Hin6I*.

15. (canceled)

16-20. (canceled)

21. (canceled)

22. (canceled)

23. (previously presented) The method of claim 1, wherein said DNA methylation comprises CpG methylation.

24. (previously presented) The method of claim 1, wherein said cancer is breast cancer.

25. (previously presented) The method of Claim 1, wherein said methylation-sensitive restriction enzyme comprises *Hin 6I*.

26. (previously presented) The method of Claim 1, further comprising the step of i) separating said plasma sample into a control sample and an experimental sample, and ii) adding control nucleic acid to both said control and experimental samples, wherein said control nucleic acid comprises at least one known CpG island that is unmethylated.

27. (previously presented) The method of Claim 26, wherein said control sample is not exposed to said digesting and said experimental sample is exposed to said digesting, and wherein both said control and experimental samples are contacted with primers specific for said control nucleic acid under conditions such that a fragment of said control nucleic acid is amplified only

if said known CpG island is uncleaved.

28. (previously presented) The method of Claim 27, further comprising comparing any fragments amplified in said control and experimental samples to confirm that said digesting in step b) is complete.

29. (cancelled)

30. (previously presented) The method of Claim 1, wherein said plurality of different promoters comprises at least 40 different promoters from at least 40 different genes.

31. (previously presented) The method of Claim 1, wherein said digesting is performed to completion.

32. (cancelled)

33. (previously presented) A method, comprising:

- a) providing a biological sample from a subject, said biological sample comprising genomic DNA, wherein said genomic DNA comprises a plurality of promoters from different genes;
- b) adding control nucleic acid to said biological sample and separating said biological sample into a control sample and an experimental sample, wherein said control nucleic acid comprises at least one known CpG island that is unmethylated;
- c) digesting said experimental sample, but not said control sample, with a methylation-sensitive restriction enzyme under conditions such that: i) unmethylated CpG islands in said promoters are cleaved while methylated CpG islands in said promoters are not cleaved, and ii) unmethylated CpG islands in said control nucleic acid are cleaved;
- d) contacting said control and experimental samples with primers specific for said control nucleic acid and detecting the presence or absence of amplification in said experimental and control samples, wherein no detectable amplification in said

experimental sample confirms digestion was complete, and wherein amplification in said control sample confirms proper amplification of uncleaved control nucleic acid;

e) contacting said experimental sample with gene specific primers, wherein said gene specific primers are configured to hybridize to said genomic DNA, and wherein said contacting is under conditions such that fragments of said plurality of promoters comprising uncleaved CpG islands are amplified, while cleaved promoters comprising cleaved CpG islands are not amplified; and

f) detecting the presence or absence of DNA methylation in each of said plurality of promoters in said experimental sample based on the amplification, or lack of amplification, of said fragments to generate a methylation profile for said subject.